CLAIMS

What is claimed is:

SUB A2

1. A method comprising introducing into a cell a sense RNA fragment of a viral genome or portion thereof and an antisense RNA fragment of said viral genome or portion thereof, wherein said sense RNA fragment and said antisense RNA fragment are capable of forming a double-stranded RNA molecule, wherein the expression of said viral genome or portion thereof in said cell is altered.

2. The method of claim 1, wherein said cell is virus resistant or tolerant.

3. The method of claim 1, wherein said cell is a plant cell.

4. The method of claim 2, wherein said virus is selected from the group consisting of topsoviruses, potyviruses, potexviruses, tobamoviruses, luteoviruses, cucumoviruses, bromoviruses, closteorviruses, tombusviruses and furoviruses.

5. The method of claim 1, wherein said RNA fragments comprises a nucleotide sequence derived from a viral coat protein gene, a viral nucleocapsid protein gene, a viral replicase gene, a movement protein gene or portions thereof.

6. The method of claim 1, wherein said RNA fragments are comprised in two different RNA molecules.

7. The method of claim 6, wherein said RNA fragments are mixed before being introduced into said cell.

8. The method of claim 7, wherein said RNA fragments are mixed before being introduced into said cell under conditions allowing said RNA fragments to form a double-stranded RNA molecule.

9. The method of claim 6, wherein said RNA fragments are introduced into said cell sequentially.

10. The method of claim 1, wherein said RNA fragments are comprised in one RNA molecule.

11. The method of claim 10, wherein said RNA molecule is capable of folding such that said RNA fragments comprised therein form a double-stranded region.

12. A method comprising introducing into a cell a first DNA sequence capable of expressing in said cell a sense RNA fragment of a viral genome or portion thereof and a second DNA sequence capable of expressing in said cell an antisense RNA fragment of said viral genome or portion thereof, wherein said sense RNA fragment and said antisense RNA fragment are capable of forming a double-stranded RNA molecule, wherein the expression of said viral genome or portion thereof in said cell is altered.

13. The method of claim 12, wherein said cell is virus resistant or tolerant.

14. The method of claim 12, wherein said cell is a plant cell.

15. The method of claim 13, wherein said virus is selected from the group consisting of topsoviruses, potyviruses, potexviruses, tobamoviruses, luteoviruses, cucumoviruses, bromoviruses, closteorviruses, tombusviruses and furoviruses.

16. The method of claim 13, wherein said DNA sequences comprises a nucleotide sequence derived from a viral coat protein gene, a viral nucleocapsid protein gene, a viral replicase gene, a movement protein gene or portions thereof.

17. The method of claim 12, wherein said first DNA sequence and said second DNA sequence are stably integrated in the genome of said cell.

18. The method of claim 12, wherein said first DNA sequence and said second DNA sequence are comprised in two different DNA molecules.

19. The method of claim 18, wherein said DNA molecules further comprise a first promoter operably linked to said first DNA sequence and a second promoter operably linked to said second DNA sequence.

20. The method of claim 12, wherein said first DNA sequence and said second DNA sequence are comprised in one DNA molecule.

21. The method of claim 20, wherein said first DNA sequence and said second DNA sequence are comprised in the same DNA strand of said DNA molecule.

22. The method of claim 21, wherein said sense RNA fragment and said antisense RNA fragment are comprised in one RNA molecule.

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23. The method of claim 22, wherein said RNA molecule is capable of folding such that said RNA fragments comprised therein form a double-stranded region.

24. The method of claim 22, wherein said DNA molecule further comprises a promoter operably linked to said first or said second DNA sequence.

25. The method of claim 24, wherein said promoter is a heterologous promoter.

26. The method of claim 24, wherein said promoter is a tissue-specific promoter.

27. The method of claim 24, wherein said promoter is a developmentally regulated promoter.

28. The method of claim 24, wherein said promoter is a constitutive promoter.

29. The method of claim 24, wherein said promoter is an inducible promoter.

30. The method of claim 22, wherein said DNA molecule further comprises a linker between the DNA sequences encoding said sense RNA fragment and said antisense RNA fragments.

31. The method of claim 30, wherein said linker comprises an expression cassette comprising a functional gene.

32. The method of claim 31, wherein said functional gene is a selectable marker gene.

33. The method of claim 31, wherein said linker comprises regulatory sequences.

34. The method of claim 33, wherein said regulatory sequences comprise intron processing signals.

35. The method of claim 21, wherein said sense RNA fragment and said antisense RNA fragment are comprised in two RNA molecules.

36. The method of claim 35, wherein said first DNA sequence is operably linked to a first promoter and said second DNA sequence is operably linked to a second promoter.

37. The method of claim 35, wherein said first DNA sequence and said second DNA sequence are operably linked to a bi-directional promoter.

38. The method of claim 21, wherein said first DNA sequence and said second DNA sequence are comprised in complementary strands of said DNA molecule.

39. The method of claim 38, wherein said first DNA sequence is the complementary DNA strand of said second DNA sequence in said DNA molecule.

40. The method of claim 39, wherein said DNA molecule further comprises a first promoter operably linked to said first DNA sequence.

41. The method of claim 40, wherein said DNA molecule further comprises a first site-specific recombination site between said first promoter and said first or second DNA sequence and a second site-specific recombination site at the 3'end of said first or second DNA sequence, wherein said first and second site-specific recombination sites are capable of inverting said first or second DNA sequence between said first and second site-specific recombination sites in presence of a site-specific recombinase.

42. The method of claim 41, wherein as a result of said inverting said first promoter is capable of expressing said second or first DNA sequence.

43. The method of claim 41, wherein said cell further comprises a site-specific recombinase capable of recognizing said site-specific recombination sites.

44. The method of claim 40, wherein said DNA molecule further comprises a second promoter operably linked to said second DNA sequence.

45. A cell comprising the sense and antisense RNA fragments of claim 1.

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46. The cell of claim 45, wherein the expression of said target gene in said cell is altered by said RNA fragments.

47. The cell of claim 46, wherein said cell is virus resistant or tolerant.

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48. The cell of claim 45, wherein said cell is a plant cell.

Sub B1

49. A plant and the progeny thereof derived from the plant cell of claim 48.

50. The plant of claim 49, wherein said plant is virus resistant or tolerant.

51. Seeds derived from the plant of claim 49.

52. A cell obtained by the method of claim 1.

53. A cell comprising the two DNA sequences of claim 12, wherein the expression of said target gene in said cell is altered, when said DNA sequences are expressed.

54. The cell of claim 53, wherein said cell is virus resistant or tolerant.

55. The cell of claim 53, wherein said cell is a plant cell.

56. A plant and the progeny thereof derived from the plant cell of claim 55.

57. The plant of claim 56, wherein said plant is virus resistant or tolerant.

58. Seeds derived from the plant of claim 56.

59. A cell obtained by the method of claim 12.

60. The cell of claim 59, wherein said cell is virus resistant or tolerant.

61. The cell of claim 60, wherein said cell is a plant cell.

62. A cell comprising the two DNA sequences of claim 8, wherein said cell further comprises a sense RNA fragment and an antisense RNA fragment of said target gene.

63. A DNA construct comprising a first DNA sequence capable of expressing in a cell a sense RNA fragment of a viral genome or portion thereof and a second DNA sequence capable of expressing in said cell an antisense RNA fragment of said viral genome or portion thereof, wherein said sense RNA fragment and said antisense RNA fragment are capable of forming a double-stranded RNA molecule.

64. The DNA construct of claim 63, wherein the expression of said viral genome or portion thereof in said cell is altered.

65. The DNA construct of claim 63, wherein said cell is a plant cell.

66. The DNA construct of claim 63, wherein said DNA construct further comprises a promoter operably linked to said first or said second DNA sequence.

67. The DNA construct of claim 63, wherein said DNA construct further comprises a first promoter operably linked to said first DNA sequence and a second promoter operably linked to said second DNA sequence.

68. The DNA construct of claim 63, wherein said DNA construct further comprises a bi-directional promoter operably linked to said first DNA sequence and to said second DNA sequence.

69. A DNA construct comprising a first DNA sequence capable of expressing in a cell a sense RNA fragment of a target gene and a second DNA sequence capable of expressing in said cell an antisense RNA fragment of said target gene, wherein said sense RNA fragment and said antisense RNA fragment are capable of forming a double-stranded RNA molecule, wherein said DNA construct further comprises a bi-directional promoter operably linked to said first DNA sequence and to said second DNA sequence.

70. The DNA construct of claim 69, wherein the expression of said target gene in said cell is altered.

71. A DNA construct comprising:

(a) a first DNA sequence capable of expressing in a cell a sense RNA fragment of a target gene and a second DNA sequence capable of expressing in said cell an antisense RNA fragment of said target gene, wherein said sense RNA fragment and said antisense RNA fragment are capable of forming a double-stranded RNA molecule, wherein said first DNA sequence is the complementary strands of said second DNA sequence in said DNA construct,

(b) a promoter operably linked to said first or second DNA sequence,

(c) a first site-specific recombination site between said promoter and said first or second DNA sequence, and

(d) a second site-specific recombination site at the 3'end of said first or second DNA sequence, wherein said first and second site-specific recombination sites are capable of inverting said first or second DNA sequence between said first and second site-specific recombination sites in presence of a site-specific recombinase.

72. A DNA construct comprising:

(a) a first DNA sequence capable of expressing in a cell a sense RNA fragment of a target gene and a second DNA sequence capable of expressing in said cell an antisense RNA fragment of said target gene, wherein said sense RNA fragment and said antisense RNA fragment are capable of forming a double-stranded RNA molecule, wherein said first DNA sequence is the complementary strands of said second DNA sequence in said DNA construct,

(b) a first promoter operably linked to said first DNA sequence,

(c) a second promoter operably linked to said second DNA sequence.

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